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Annulohypoxylon annulatoides newly described in Taiwan

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ABSTRACT

Annulohypoxylon annulatoides is described as new, being characterized by having thick stromata with a dark, olivaceous-tinged surface, *truncatum*-type ostiolar discs measuring 0.4–0.5 mm diam, and a conidiogenous structure with a nodulisporium-like branching pattern. It bears resemblance to *A. annulatum*, a species mainly distributed in North America and temperate Asia, from which *A. annulatoides* differs by having pulvinate to effused-pulvinate rather than hemispherical stromata, less convex ostiolar discs, and a more conspicuous ostiolar disc rim. The distinctiveness between *A. annulatoides* and *A. annulatum* are reconfirmed by ITS sequences.

Key words: Ascomycota, Hypoxyloideae, *Hypoxylon*, taxonomy, wood-dwelling, Xylariaceae

Introduction

Annulohypoxylon Y.-M. Ju, J.D. Rogers & H.-M. Hsieh was established by Hsieh et al. (2005) for the taxa included in *Hypoxylon* Bull. sect. *Annulata* J. H. Miller sensu Ju and Rogers (1996). *Annulohypoxylon* differs from *Hypoxylon* primarily in having ostioles encircled with a truncate area, carbonized stromatal tissue discretely enclosing each individual perithecium, and a thickening on the ascospore perispore located at approximately one-third of the spore length from one end (Ju and Rogers 1996). In contrast, the ascospore perispores for most *Hypoxylon* species have conspicuous or inconspicuous coil-like ornamentation. *Annulohypoxylon truncatum* (Schwein.) Y.-M. Ju, J. D. Rogers & H.-M. Hsieh, the type species of the genus, was considered by Miller (1961) [as *Hypoxylon truncatum* (Schwein.) J. H. Miller] to have a worldwide distribution. Miller's broad species concept was formed by lumping many species together, as reflected by the long list of synonyms for *H. truncatum* in Miller (1961), where ostiolar discs are 0.3–0.5 mm diam and ascospores fall in the size range of 7–9 × 3–4 μm. Ju and Rogers (1996) segregated *A. annulatum* (Schwein.) Y.-M. Ju, J. D. Rogers & H.-M. Hsieh, *A. moriforme* (Henn.) Y.-M. Ju, J. D. Rogers & H.-M. Hsieh, and *A. nitens* (Ces.) Y.-M. Ju, J. D. Rogers & H.-M. Hsieh as distinct species from *A. truncatum*. *Annulohypoxylon truncatum* and *A. annulatum*, as defined by Ju and Rogers (1996), are mainly distributed in North America and temperate Asia with *Quercus* species.

We collected an *Annulohypoxylon* species, which is newly described herein as *A. annulatoides*. It resemble *A. annulatum*, a species highly associated with fallen *Quercus* branches and frequently mixed with *A. truncatum*. However, *A. annulatoides* has more flattened stromata, a more conspicuous ostiolar disc rim on mature stromata, and

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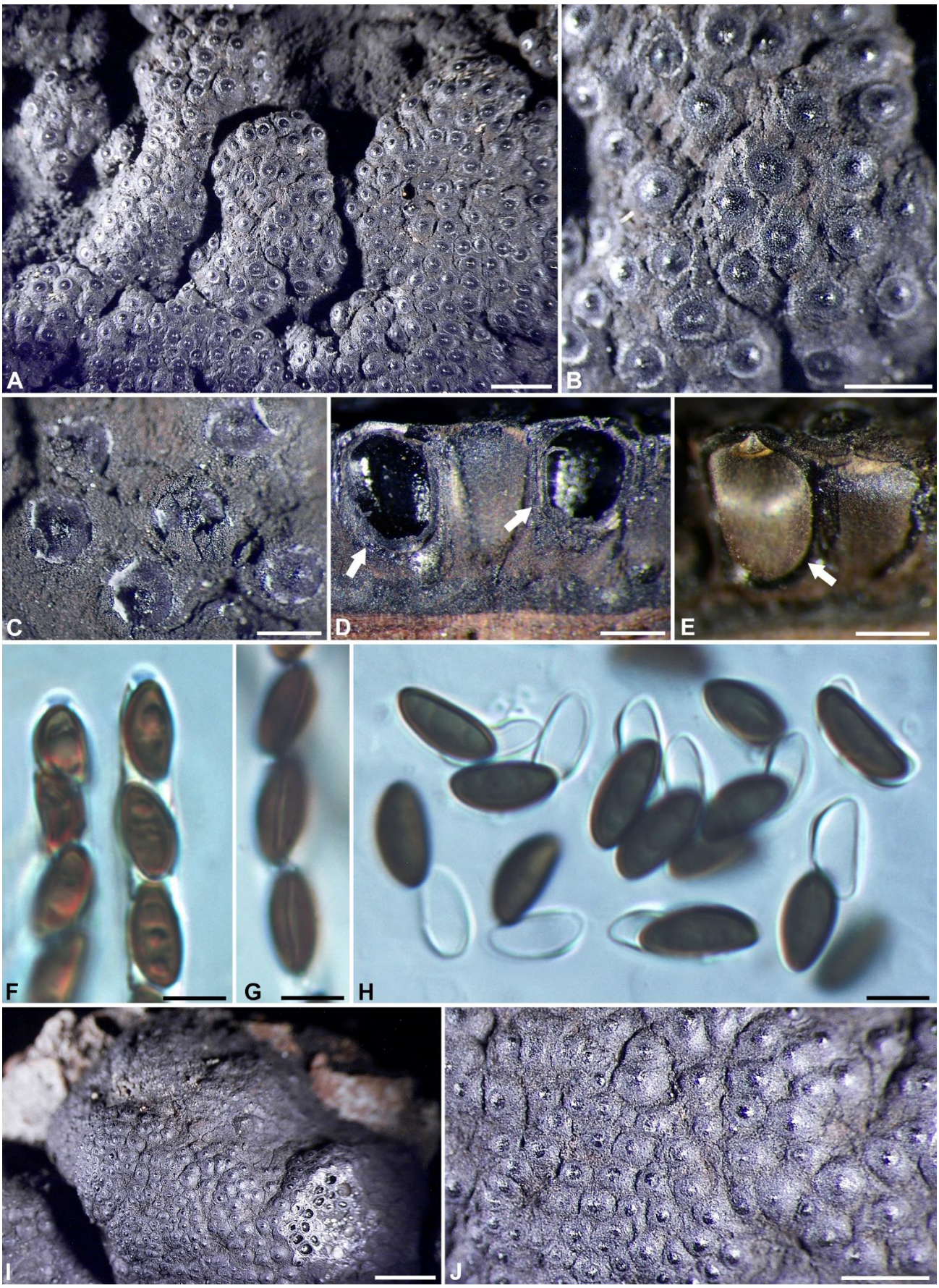
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a geographical distribution in Taiwan, a subtropical island.

Materials and Methods

Asci, ascospores, conidiogenous cells, and conidia were examined using differential interference contrast microscopy and bright field microscopy. Material was mounted in water for examination, while Melzer's iodine reagent and 10% KOH solution were used to test the amyloid reaction of the ascal apical rings and the dehiscence of the perispore, respectively. The color designations follow Rayner (1970).

Cultures were obtained by placing pieces of tissue of freshly collected stromata on malt extract agar without peptone added as given in Kenerley and Rogers (1976). Resulting colonies were transferred to 9-cm plastic Petri dishes containing 2% oatmeal agar (OA), from which the culture description was made, and incubated at 20°C under 12 hours of fluorescent light. Cultures were deposited at BCRC (Bioresource Collection and Research Center, Hsinchu, Taiwan).

Total DNA was extracted from dried mycelia following Ju et al. (2022). PCR amplification of ITS was described in Hsieh et al. (2009). ITS sequences were subjected to NCBI MEGABLAST queries.

Taxonomy

Annulohypoxylon annulatoides Y.-M. Ju & H.-M. Hsieh, sp. nov. Figs. 1, 2

MycoBank MB856526

Etymology. Referring to its resemblance to *A. annulatum*, from which the stromata of the present species are pulvinate to effused-pulvinate rather than hemispherical, and the ostiolar disc rim is more conspicuous.

Typification. TAIWAN. Nan-tou County, Yu-chee, Lien-hwa-chee, on corticated wood of *Machilus zuihoensis*, 12 Mar 2003, *Ju, Y.-M. & Hsieh, H.-M. 92031202* (cultured from stroma *YMJ2247*) (holotype HAST 146305), ITS sequence deposited at GenBank as PQ590625.

Stromata pulvinate to effused-pulvinate, 0.6–6 cm long × 0.5–2.5 cm broad × 1–1.5 mm thick; surface dull black, tinged olivaceous, with perithecial mounds inconspicuous to 1/3 exposed, overlain with a dark olivaceous, irregularly cracked layer on maturing stromata but entirely worn off on fully mature stromata, dark brown to blackish granules immediately beneath surface, with KOH-extractable pigments Dull Green (70); tissue between perithecia dark brown, woody, with carbonaceous tissue encasing each perithe-

Fig. 1. *Annulohypoxylon annulatoides* and *A. annulatum*. A–H. *A. annulatoides* (from holotype). A. Stromata. B. Fully mature stromatal surface showing coarsely papillate ostiolar openings that are individually encircled with a truncate disc with a distinct rim. C. Stromatal surface showing developing ostiolar discs in various stages. D. Vertical section of perithecia encased in a layer of carbonaceous tissue (arrows). E. Perithecia revealed by partially removing the carbonaceous encasing tissue (arrow). F. Ascal apical ring staining light blue in Melzer's iodine reagent and ascospores. G. Ascospores showing a germ slit. H. Ascospores with perispores dehiscing in 10% KOH. I, J. *A. annulatum* (from HAST 146311). I. Stromata. J. Fully mature stromatal surface showing coarsely papillate ostiolar openings that are individually encircled with a truncate disc with a vague rim. Bars in A, I = 2 mm; B, J = 1 mm; C–E = 0.5 mm; F–H = 5 μm.

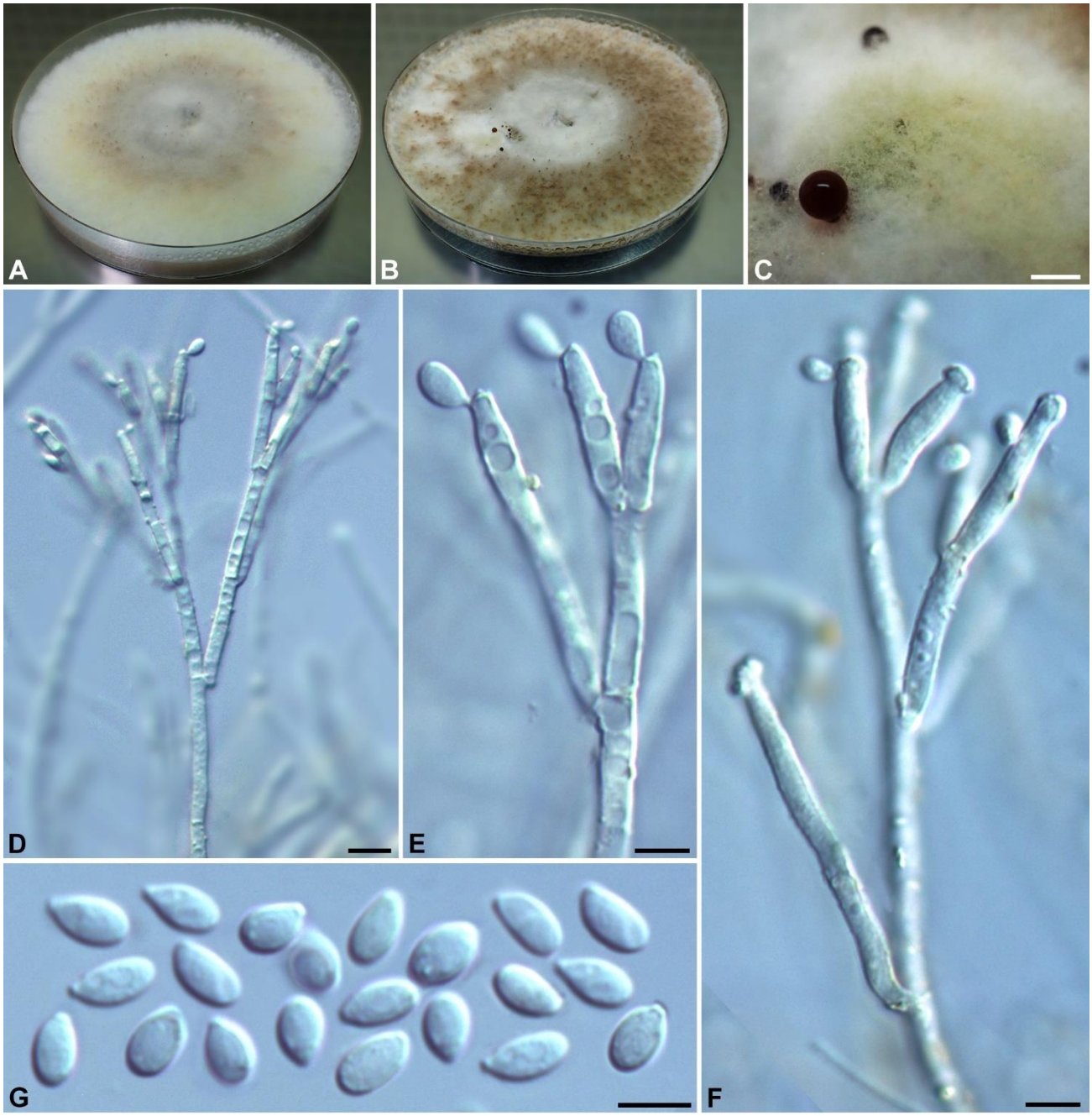

Fig. 2. *Annulohypoxylon annulatoides* (from holotype). A, B. Colony on a 9-cm Petri plate containing OA at 2 wk and 3 wk, respectively. C. Sporulating area on OA with dark brown exudates. D–F. Conidiophores; conidiogenous cells in F with the apical region becoming swollen after multiple conidia have been produced. G. Conidia. Bars in C = 2 mm; D = 10 µm; E–G = 5 µm.

cium; tissue below perithecial layer 0.1–0.3 mm thick, carbonaceous. Perithecia ellipsoid to obovoid, 0.6–0.8 mm diam × 0.8–1 mm high. Ostioles higher than stromatal surface, with ostiolar openings coarsely papillate, encircled with a flattened to slightly convex *truncatum*-type disc as defined in Ju and Rogers (1996), 0.4–0.5 mm diam. Asci with eight ascospores arranged in uniseriate manner, cylindrical, 130–180 µm total length, the spore-bearing part 60–75 µm long ×

4.5–6 µm broad, with an apical ring staining pale blue to light blue in Melzer's iodine reagent, discoid, 0.8–1.0 µm high × 1.7–2.2 µm broad. Ascospores light brown to brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, smooth, (7.4–)8.1–9.1(–10.2) × (3.6–)3.8–4.3(–4.6) µm (8.6 ± 0.5 × 4.0 ± 0.2 µm, N = 80), with a straight germ slit spore-length on the dorsal side, perispore dehiscent in 10% KOH, smooth, with a thickened area visible at the position of approximately 1/3 ascospore length from one end on the same side as the germ slit; epispore smooth.

Cultures and anamorph. Colonies reaching the edge of 9-cm Petri dish in 1.5–2 wk, whitish initially, immediately becoming Amber (47) and quickly turning into Umber (9) from center outwards, felty, faintly zonate, sometimes with Dark Brick (60) to Sepia (63) exudates, with diffuse margins. Reverse Olivaceous (48) to Dull Green (48). Sporulating regions in Amber (47) to Honey (64) patches. Conidiogenous structure upright, mononematous, with nodulisporium-like branching pattern, 3–3.5 µm broad at main axis, smooth or slightly roughened, hyaline to yellowish. Conidiogenous cells 2–5 arising from each terminal branch, cylindrical, 11–20 × 2.5–3.5 µm, smooth or finely roughened, bearing poroid conidial secession scars on apical region, with apical region becoming swollen after multiple conidia produced. Conidia produced holoblastically in sympodial sequence, hyaline, smooth, obovoid to ellipsoid, equilateral to slightly inequilateral, (4.3–)4.7–5.5(–6.0) × (2.7–)3.0–3.4 (–3.9) µm (5.1 ± 0.4 × 3.2 ± 0.2 µm, N = 80), with a minute flattened base indicating former point of attachment to conidiogenous cell.

Other specimens examined. TAIWAN. I-lan County, Yuan-shan, Fu-shan, on corticated wood, 11 Oct 2000, *Ju, Y.-M. & Hsieh, H.-M. 89101104* (cultured from stroma *YMJ2246*) (HAST 146302), ITS sequence deposited at GenBank as PQ590626; I-lan County, Yuan-shan, Fu-shan, on corticated wood, 4 Sep 2003, *Ju, Y.-M. & Hsieh, H.-M. 92090411* (cultured from stroma *YMJ2250*) (HAST 146303), ITS sequence deposited at GenBank as PQ590627; Miao-li County, Nan-zhuang Township, Dong-he Village, Shi-man Historical Trail, on corticated wood, 9 Oct 2011, *Guu, J.-R. 100100903* (cultured from stroma *YMJ2252*) (HAST 146304), ITS sequence deposited at GenBank as PQ590628; Ping-tung County, Heng-chun, Ken-ting, on corticated wood, 16 Sep 2000, *Ju, Y.-M. & Hsieh, H.-M. 89091635* (cultured from stroma *YMJ2245*) (HAST 146306), ITS sequence deposited at GenBank as PQ590629; Ping-tung County, Heng-chun, Ken-ting, on corticated wood, 20 Jul 2003, *Ju, Y.-M. & Hsieh, H.-M. 92072002* (cultured from stroma *YMJ2249*) (HAST 146307), ITS sequence deposited at GenBank as PQ590630; Taipei City, Nan-kang District, Hu-shi Park, on corticated wood, 6 Apr 2003, *Ju, Y.-M. & Hsieh, H.-M. 92040601* (cultured from stroma *YMJ2248*) (HAST 146308), ITS sequence deposited at GenBank as PQ590631; Taipei City, Nan-kang District, Nan-kang-shan Trail, on corticated wood, 4 Jul 2006, *Guu, J.-R. 95070401* (cultured from stroma *YMJ2251*) (HAST 146309), ITS sequence deposited at GenBank as PQ590632.

Specimens of *A. annulatum* examined. JAPAN. Kanagawa Prefecture, Odawara, Iryuda, on corticated wood, 9 Dec 2018, *Ju, Y.-M. & Hsieh, H.-M.* (HAST 146310), ITS sequence deposited at

GenBank as PQ590633. USA. Florida, Gainesville, on corticated wood of *Quercus* sp., Aug 1972, *Rogers, J. D. & Kimbrough, J. W.* (HAST 146311), ITS sequence deposited at GenBank as PQ590634.

Notes. *Annulohypoxylon annulatoides* closely resembles *A. annulatum* in having thick stromata with a dark, olivaceous-tinged surface and *truncatum*-type ostiolar discs measuring 0.4–0.5 mm diam. Microscopically, *A. annulatoides* is also similar to *A. annulatum*, with ascospore measuring 7.5–10.5(–11) × 3.5–5(–6) µm and conidia measuring 4.5–6 × 2.5–3 µm, produced on a conidiogenous structure with a nodulisporium-like branching pattern (Ju and Rogers 1996).

Their differences, although subtle, are consistent. The ostiolar discs of *A. annulatoides* are less convex compared to those of *A. annulatum*. Unlike *A. annulatoides*, the ostiolar disc rim in *A. annulatum* eventually becomes quite inconspicuous. While *A. annulatum* commonly produces hemispherical stromata, *A. annulatoides* frequently produces pulvinate to effused-pulvinate stromata.

ITS sequences of the eight cited specimens of *A. annulatoides* showed pairwise similarities ranging from 99.2% to 99.9%. In contrast, these ITS sequences shared only 86.9% to 87.5% similarities with those of the two cited specimens of *A. annulatum*.

The geographic distribution of *A. annulatum* is primarily in North America and temperate Asia, commonly associated with *Quercus* species. The host range of *A. annulatoides* remains uncertain. One of the specimens that we studied was collected from *Machilus zuihoensis* (Lauraceae), and others were from corticated wood of unidentified tree species.

The specimens of *H. annulatum* reported from Taiwan by Ju and Rogers (1999) are actually *A. annulatoides*, as are those of *H. truncatum* reported in (Ju and Tzean 1985).

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References

Hsieh H-M, Ju Y-M, Hsueh P-R, Lin H-Y, Hu F-R, Chen W-L. 2009. Fungal keratitis caused by a new filamentous hyphomycete *Sagenomella keratitidis*. Botanical Studies 50:331–335.

Hsieh H-M, Ju Y-M, Rogers JD. 2005. Molecular phylogeny of *Hypoxylon* and closely related genera. Mycologia 97:844–865.

Ju Y-M, Hsieh H-M, He X-S. 2022. Wulingshen, the massive *Xylaria* sclerotia used as traditional Chinese medicine, is produced by multiple species. Mycologia 114:175–189.

Ju Y-M, Rogers JD. 1996. A revision of the genus *Hypoxylon*. St. Paul, Minnesota: APS Press, the American Phytopathological Society. xv + 365 p.

Ju Y-M, Rogers JD. 1999. The Xylariaceae of Taiwan (excluding *Anthostomella*). Mycotaxon 73:343–440.

Ju Y-M, Tzean S-S. 1985. Investigations of Xylariaceae in Taiwan I. The teleomorph of *Hypoxylon*. Transactions of the Mycological Society of Republic of China 1:13–27.

Kenerley CM, Rogers JD. 1976. On *Hypoxylon serpens* in culture. Mycologia 68:688–691.

Miller JH. 1961. A monograph of the world species of *Hypoxylon*. Athens: University of Georgia Press. xii + 158 p.

Rayner RW. 1970. A mycological colour chart. Kew, UK: Commonwealth Mycological Institute. 34 p + 2 charts.

在臺灣發現的截頂炭團菌屬新種：擬環紋截頂炭團菌

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摘　要

由臺灣多處採集到的一種截頂炭團菌屬，在此被描述為新種，並命名為擬環紋截頂炭團菌。其子座厚實、具有帶有橄欖色調的暗色表面，環繞孔口圓盤為 *truncatum*-type、直徑 0.4–0.5 mm，分生孢子產孢構造具有 nodulisporium-like 的分叉模式。環紋截頂炭團菌非常類似擬環紋截頂炭團菌，但主要分佈在北美與溫帶亞洲。擬環紋截頂炭團菌與其相異之處在於具有墊狀或闊墊狀、而不是半圓形的子座，較不凸起的孔口圓盤，以及較顯著的孔口圓盤邊緣。ITS 序列再次證實了擬環紋截頂炭團菌與環紋截頂炭團菌之間的獨特性。

關鍵詞：子囊菌門、炭團菌亞科、炭團菌屬、分類學、木棲性、炭角菌科